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# Rho GTPase signaling complexes in cell migration and invasion

Campbell D. Lawson and Anne J. Ridley

Randall Centre for Cell and Molecular Biophysics, King's College London, London, England, UK

Cell migration is dependent on the dynamic formation and disassembly of actin filament-based structures, including lamellipodia, filopodia, invadopodia, and membrane blebs, as well as on cell–cell and cell–extracellular matrix adhesions. These processes all involve Rho family small guanosine triphosphatases (GTPases), which are regulated by the opposing actions of guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Rho GTPase activity needs to be precisely tuned at distinct cellular locations to enable cells to move in response to different environments and stimuli. In this review, we focus on the ability of RhoGEFs and RhoGAPs to form complexes with diverse binding partners, and describe how this influences their ability to control localized GTPase activity in the context of migration and invasion.

## Introduction

Cell migration is essential for animal development and physiology, and is also associated with pathophysiological processes, such as chronic inflammation and cancer metastasis. Cells migrate *in vitro* and *in vivo* either as single cells or as groups or sheets, known as collective migration (De Pascalis and Etienne-Manneville, 2017; Friedl and Mayor, 2017). At the leading edge of single cells, such as immune cells, and cell groups, such as sprouting blood vessels, cells often extend lamellipodia and filopodia, in which the plasma membrane is driven forward by actin polymerization (Fig. 1 A; Ridley, 2015). Localized actomyosin contractility is also required at both the front and rear of the cell. The dynamic formation and disassembly of all of these actin-based structures allow the cell to fine-tune its direction of migration in response to extracellular cues. In addition, cell–cell and cell–extracellular matrix adhesions rapidly turn over to permit cell movement across and through tissues.

Alternatively, both single cells and cells at the edge of tissues *in vivo* can migrate using bleb-based forward protrusion, in which the plasma membrane transiently detaches from the cortical actin network, and the protrusion is then stabilized by actin polymerization (Fig. 1 B; Paluch and Raz, 2013). Blebbing is usually associated with a high level of actomyosin contractility in cells, which again needs to be dynamically regulated to allow changes in cell directionality. Bleb-based migration is observed in some cell types during development and in several cancer cell lines in 3D matrices and/or *in vivo*.

To migrate through tissues *in vivo*, cells often have to degrade the ECM, and this involves specialized structures known as invadopodia and podosomes (Paterson and Courtneidge, 2017). These are actin-rich protrusions that are dependent on actin-regulatory proteins such as WASL (N-WASP), cortactin, and cofilin for their assembly. Transmembrane and secreted metalloproteases are specifically delivered to invadopodia, which degrade ECM proteins locally and thereby contribute to cell invasion (Fig. 1 A).

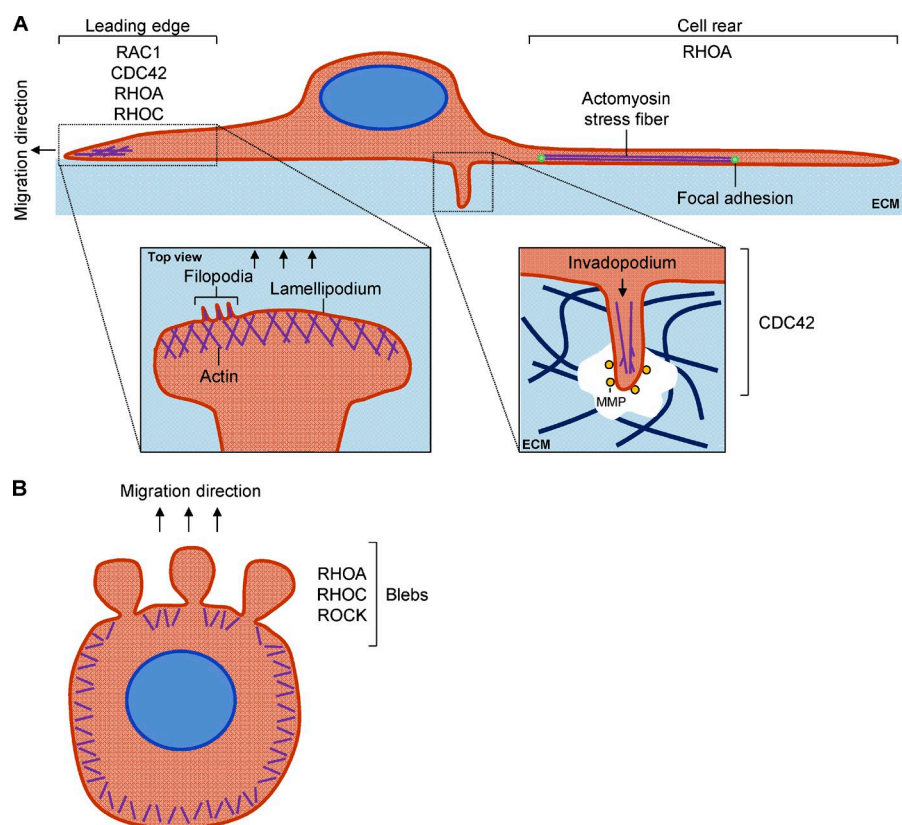
Efficient migration and/or invasion requires the coordinated dynamics of the cellular components described (lamellipodia, filopodia, cell–cell adhesions, cell–extracellular matrix adhesions, membrane blebs, and/or invadopodia), and these structures are therefore tightly regulated by multiple signaling mechanisms. In particular, members of the Rho family of small GTPases have been shown to play essential roles in cell migration and invasion through the regulation of these processes, acting at specific locations and times in cells (Fig. 1 and Fig. 2 A; Fritz and Pertz, 2016).

The 20 members of the Rho family can be divided into classic and atypical members (Fig. 2 A). Classic Rho GTPases, such as RHOA, RAC1, and CDC42, are regulated by the opposing actions of Rho-specific guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs; Fig. 2 B). RhoGEFs activate Rho GTPases by stimulating the exchange of a bound GDP nucleotide for GTP, whereas RhoGAPs catalyze GTP hydrolysis, thus returning these proteins to an inactive state (Bos et al., 2007). Atypical Rho family members include the Rnd subfamily and RHOH, which are unable to hydrolyze GTP and are therefore constitutively GTP-bound, and RHOU, which has a high intrinsic nucleotide exchange rate and hence is predicted to be predominantly GTP-bound in cells (Haga and Ridley, 2016). Most Rho GTPases are modified at their C-termini by isoprenyl lipids, which facilitate their localization to membranes (Mitin et al., 2012). Rho guanine nucleotide dissociation inhibitors (GDIs) regulate RHOA, RAC1, and CDC42 by binding to isoprenyl groups and thereby extracting them from membranes. In addition to GTP/GDP cycling, Rho GTPases are regulated by posttranslational modifications, including phosphorylation and ubiquitylation (Hodge and Ridley, 2016).

Many GEFs and GAPs have been reported to contribute to Rho GTPase-mediated migration (Tables S1 and S2; Goicoechea et al., 2014; Lawson and Burridge, 2014; Hodge and Ridley, 2016). However, the dynamic regulation of Rho GTPases

Correspondence to Anne J. Ridley: anne.ridley@kcl.ac.uk





**Figure 1. Rho GTPase-driven single cell migration modes.** (A) Individual cells can migrate in a lamellipodium-based manner with actin polymerization (shown in purple) driving formation of lamellipodia and filopodia at the front of the cell, and actomyosin contractility promoting retraction at the cell rear. Invasive cells can also degrade the ECM via the action of secreted matrix metalloproteinases (MMPs) that are delivered to invadopodia. The Rho GTPases involved at each of these regions are indicated. (B) Alternatively, cells can migrate in a bleb-driven manner, which is characterized by high levels of Rho/ROCK activity and actomyosin contractility.

needed for cells to migrate in response to changes in their environment requires the coordinated and localized activation/inactivation of multiple components, rather than a simple linear interaction first between a GEF and a Rho GTPase, then between the Rho GTPase and its effector target, and finally between a GAP and the Rho GTPase (Fig. 2 B). Here we will focus on the ability of RhoGEFs and RhoGAPs to form complexes with a variety of other proteins, and how these complexes regulate cell migration and invasion by determining when and where Rho GTPases are activated in cells, through a process known as spatiotemporal activation.

### GEF complexes

There are two subtypes of RhoGEF, the Dbl family and the DOCK family, and members of both can contribute to cell migration (Table S1; Cook et al., 2014; Gadea and Blangy, 2014). There are around 70 human Dbl family GEFs, all of which contain a Dbl-homology domain that stimulates guanine nucleotide exchange and is usually flanked by a pleckstrin-homology (PH) domain. The role of the PH domain varies considerably between different Dbl family GEFs. PH domains can bind to phosphoinositides or other phospholipids, thereby contributing to membrane localization in the vicinity of membrane-associated Rho GTPases. These domains can also act more directly to promote GEF–Rho GTPase interaction and/or nucleotide exchange (Rossman et al., 2005).

The domain structure of the 11 human DOCK family GEFs differs from that of the Dbl family in that, instead of a Dbl homology–PH tandem domain, they have a DOCK-homology region 2 (DHR2) domain, which stimulates guanine nucleotide exchange, and a DHR1 domain, which interacts with phospholipids and helps to target DOCK GEFs to the plasma membrane (Laurin and Côté, 2014).

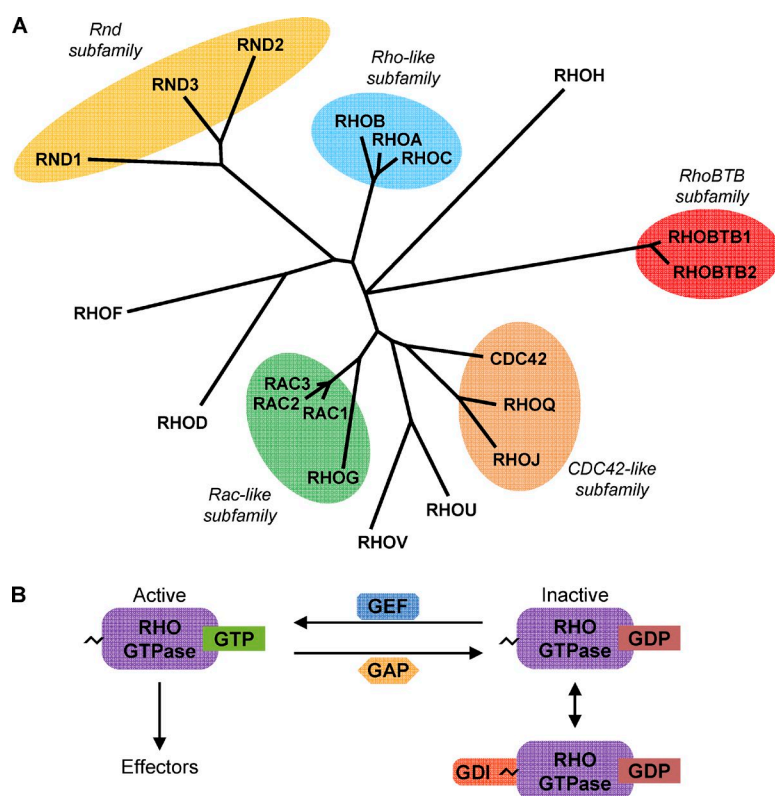
Both Dbl and DOCK family RhoGEFs also contain a variety of other domains that are specific for each subfamily within each group (Cook et al., 2014; Laurin and Côté, 2014). For example, several GEFs have SH2 and/or SH3 domains. These additional domains enable different GEFs to form specific protein complexes, which contribute to migration and invasion in distinct ways, often dependent on the cell type and the combination of internal and external stimuli. Spatiotemporal Rho GTPase activation is mediated by RhoGEFs complexed to diverse proteins such as cytoskeletal or focal adhesion components, adaptors, Rho GTPase effectors, or even to RhoGAPs.

Here, we describe GEFs for the Rac, CDC42, and Rho subfamilies of GTPases (Fig. 2 A) that contribute to cell migration through these different signaling complexes.

**Rac/CDC42-specific Dbl family GEF complexes.** Rac and CDC42 have multiple functions in cell migration and invasion that range from stimulating actin polymerization at the leading edge of cells to regulating invadopodial turnover and stability, as well as cell–cell and cell–extracellular matrix contacts (Fig. 1 A). Several GEFs are able to activate Rac and/or CDC42 (Cook et al., 2014), yet they influence migration in different ways, reflecting the requirement for Rac and CDC42 to be activated at different cellular locations together with a distinct subset of their downstream targets (Table S1 and Fig. 3).

**$\beta$ -PIX.** The Dbl family GEF ARHGEF7 ( $\beta$ -PIX) is one of the most extensively studied for its roles in cell migration and invasion, and can form several different types of protein complexes (Fig. 3 A), some of which activate Rac and others CDC42.  $\beta$ -PIX can influence migration in different ways depending on the interactions it forms and its spatial distribution (Fig. 3 A).

Perhaps the best known signaling unit involving  $\beta$ -PIX is the trimolecular GIT– $\beta$ -PIX–PAK complex, which promotes



**Figure 2. The Rho GTPase family.** (A) Unrooted phylogenetic tree representing the relationship between the 20 human Rho GTPase family members based on their sequence identity. Primary amino acid sequences were aligned using BLAST software (National Institutes of Health) and the tree constructed using TreeView (University of Glasgow). (B) Diagram of classic Rho GTPase regulation by GEFs, GAPs, and GDIs. GEFs activate Rho GTPases by stimulating the exchange of a bound GDP nucleotide for GTP, whereas RhoGAPs inactivate Rho GTPases by catalyzing GTP hydrolysis. GDIs bind to the isoprenyl groups on RHOA, RAC1, and CDC42 and thereby extract them from membranes. See text for further details.

RAC1 activity and cell migration upon interaction of GIT with paxillin in integrin-containing focal adhesions (Frank and Hansen, 2008). GIT itself is a GAP for the Arf family of GTP-binding proteins, and thus the complex combines Rac activation with Arf inactivation, which may be important for its function in cell migration (Zhou et al., 2016). PAKs are protein kinases that are effectors for RAC1 and CDC42, and thus  $\beta$ -PIX is an example of a GEF that can directly couple its associated GTPases to a specific effector (Manser et al., 1998; Radu et al., 2014). The GIT/ $\beta$ -PIX complex localizes to small integrin-based adhesions near the leading edge of migrating cells in response to phosphorylation of paxillin by PAK, as part of a positive feedback loop involved in regulating adhesion assembly and disassembly (Nayal et al., 2006). The GIT- $\beta$ -PIX-PAK complex also interacts with another Rho family member, RHOJ (Fig. 2), which acts similarly to RAC1 in stimulating focal adhesion turnover (Wilson et al., 2014).

In addition to its recruitment with GIT to paxillin in focal adhesions,  $\beta$ -PIX has been shown to localize to the leading edge of migrating cells through an interaction with the scaffolding protein SCRIB. In astrocytes,  $\beta$ -PIX binds to SCRIB during scratch wound-induced migration and activates CDC42 upon its recruitment to the leading edge. This, in turn, affects cell polarization (Osmani et al., 2006).  $\beta$ -PIX interaction with SCRIB has also been observed at the leading edge of heregulin-stimulated breast cancer cells, where it influences protrusion formation via PAK (Nola et al., 2008). SCRIB is not found in focal adhesions (Osmani et al., 2006; Nola et al., 2008), indicating that the  $\beta$ -PIX-containing complexes that form at the leading edge of migrating cells are distinct from those found at focal adhesions.

Through association with the cell-cell adhesion molecule P-cadherin,  $\beta$ -PIX can also localize to cell-cell adhesions, and this interaction promotes collective cell migration via CDC42 (Plutoni et al., 2016). Indeed,  $\beta$ -PIX was identified in a screen

for RhoGEFs that are specifically required for collective cell migration (Zaritsky et al., 2017).

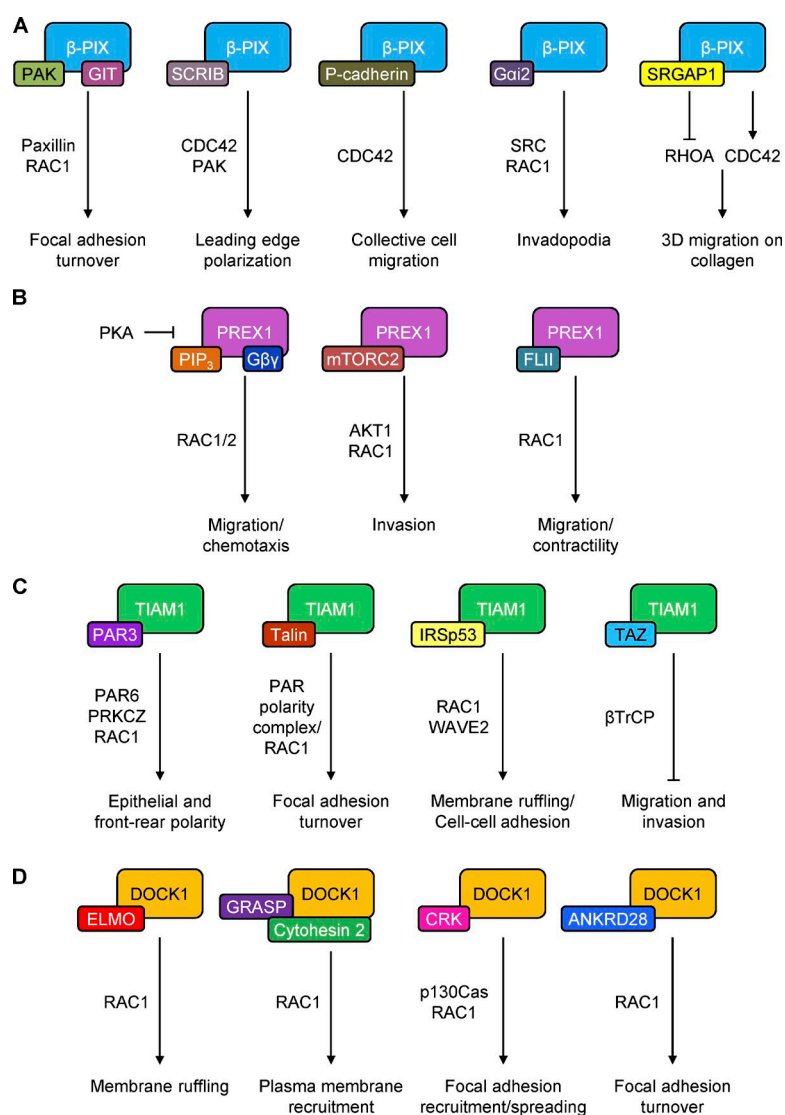
In the context of invasion, lysophosphatidic acid stimulation of its G protein-coupled receptor (GPCR) on ovarian cancer cells induces  $\beta$ -PIX to localize to invadopodia, where it forms a complex with the tyrosine kinase SRC and the heterotrimeric G protein subunit G $\alpha$ i2 (Ward et al., 2015). This interaction activates RAC1, but whether this complex alters invadopodial dynamics has not been addressed.

Finally,  $\beta$ -PIX can be part of a complex with the RhoGAP SRGAP1, and together these proteins control the 3D migration of cells in a matrix-dependent manner (Kutys and Yamada, 2014). On fibrillar collagen,  $\beta$ -PIX directly activates the Rho GTPase CDC42, whereas RHOA activity is decreased by SRGAP1. This coordinated regulation of Rho GTPases is induced by the collagen-binding  $\alpha_2\beta_1$  integrin, which stimulates  $\beta$ -PIX activity and  $\beta$ -PIX/SRGAP1 interaction as a result of dephosphorylation of  $\beta$ -PIX by the phosphatase PP2A. Knockdown of  $\beta$ -PIX or SRGAP1 causes cell rounding and motility defects on collagen matrices, highlighting the importance of this GEF-GAP association. Interestingly, the  $\beta$ -PIX/SRGAP1/CDC42/RHOA signaling pathway is not observed in cells migrating on fibronectin (Kutys and Yamada, 2014). Hence, this study not only demonstrates that different Rho GTPases are regulated differentially by the same protein complex but also shows that this  $\beta$ -PIX/SRGAP1 interaction is initiated by specific extracellular cues.

Overall, studies on  $\beta$ -PIX show that it is capable of interacting with multiple proteins, which mediate the diverse localizations and functions of this GEF in the context of migration (Fig. 3 A).

**PREX1.** The Rac-like subfamily-specific GEF PREX1 (which has also been shown to have activity toward CDC42 and RHOQ in vitro, but not in cells; Table S1) contributes to





**Figure 3. Rac/CDC42-specific GEFs form distinct complexes to differentially control cell migration and invasion.** The Rac/CDC42-specific GEFs  $\beta$ -PIX (A), PREX1 (B), TIAM1 (C), and DOCK1 (D) regulate migration in different ways depending on the complexes they form. GEFs and GEF-binding proteins are shown in arbitrarily colored boxes. For each complex, the downstream signaling targets are shown as well as the functional outcome. See text for further details.

the migration of a variety of cell types, and can be directly and synergistically activated by phosphatidylinositol (3,4,5)-trisphosphate and G $\beta$  $\gamma$  heterotrimeric G protein subunits (Fig. 3 B; Welch, 2015). Phosphatidylinositol (3,4,5)-trisphosphate- and G $\beta$  $\gamma$ -mediated PREX1 activation is inhibited by protein kinase A (PKA; Mayeenuddin and Garrison, 2006). PKA phosphorylates PREX1, and a nonphosphorylatable PREX1 mutant is able to overcome PKA-mediated inhibition of endothelial cell chemotaxis (Chávez-Vargas et al., 2016).

In addition to G $\beta$  $\gamma$ , several other PREX1-binding proteins influence its ability to promote migration (Fig. 3 B). For example, PREX1 can bind to the mammalian target of rapamycin (mTOR)-containing complexes mTORC1 and mTORC2, which are best known for their roles in cell growth and metabolism (Saxton and Sabatini, 2017). PREX1 interaction with mTORC2 is involved in leucine-induced RAC1 activation and cell migration (Hernández-Negrete et al., 2007). Furthermore, this PREX1-mTORC2 complex promotes IGF-1-stimulated ovarian carcinoma cell invasion by activating the kinase AKT1. Because constitutively active AKT1 could not stimulate migration in PREX1-depleted cells, it appears that PREX1 also acts downstream of AKT1, indicative of a positive feedback loop (Kim et al., 2011).

A recent study demonstrated that PREX1 can promote migration via FLII, a RAC1 effector that regulates the actin cytoskeleton (Marei et al., 2016). FLII can bind to both PREX1 and RAC1 via different domains, and promotes fibroblast migration in part by increasing myosin II activity and cell contractility. The mechanism through which it induces contractility is unclear, but does not appear to involve Rho-ROCK activity. Nevertheless, these findings emphasize that GEFs can directly couple their GTPase targets to specific effectors.

**TIAM1.** TIAM1 is an example of a Rac-specific GEF that has distinct functions in cell migration depending on the context and cell type. On the one hand, by enhancing cadherin-mediated cell-cell adhesion, TIAM1 can promote epithelial-like cell morphology and thereby inhibit cell migration and invasion (Hordijk et al., 1997; Marei et al., 2016). On the other hand, it can contribute to lamellipodium extension and stimulate migration (Connolly et al., 2005; Pegtel et al., 2007).

TIAM1 directs specific upstream signals to different RAC1-dependent signaling pathways through its interactions with scaffolding proteins (Rajagopal et al., 2010). For example, TIAM1 influences cell polarity as a result of interactions with the PAR polarity complex protein PAR3 (Fig. 3 C; Nishimura et al., 2005). This interaction is associated with the generation

of apical-basal epithelial polarity, and hence promotes epithelial cell–cell adhesion. However, it can also contribute to the front-rear polarity of migrating cells, as shown in persistently migrating keratinocytes (Pegtel et al., 2007; Mack and Georgiou, 2014). Furthermore, the PAR complex influences the recruitment of TIAM1 to talin, a focal adhesion protein that links integrins to the actin cytoskeleton. The binding of TIAM1 to talin controls adhesion turnover and migration of glioma cells on fibronectin (Wang et al., 2012).

TIAM1 links activated RAC1 to its effector WASF2 (WAVE2) in lamellipodia through mutual interactions with the adaptor protein BAIAP2 (IRSp53; Fig. 3 C; Connolly et al., 2005). Surprisingly, knockdown of TIAM1 or IRSp53 enhances fibroblast migration, suggesting that the cell ruffling induced by this complex leads to increased cell–cell adhesion rather than sustained migration (Rajagopal et al., 2010). It would be interesting to know if this effect involves TIAM1 interaction with PAR3. TIAM1 also inhibits migration by interacting with the TAZ/YAP transcriptional coactivators as well as with a ubiquitin ligase,  $\beta$ TrCP, which promotes TAZ/YAP degradation (Fig. 3 C; Diamantopoulou et al., 2017). TIAM1 thereby inhibits transcription of TAZ/YAP-dependent genes, which include those involved in cancer cell migration and invasion.

**VAV1 and VAV2.** CDC42 is well-known to induce filopodial protrusions, and also plays an essential role in invadopodium formation (Fig. 1 A; Yamaguchi et al., 2005). In pancreatic cancer cells, the GEF VAV1 activates CDC42 to induce assembly of invadopodia (Razidlo et al., 2014). VAV1 activation in this context is dependent on phosphorylation by SRC, a tyrosine kinase that is strongly linked to invadopodial induction (Razidlo et al., 2014; Foxall et al., 2016).

The Rac subfamily member RAC3 has also recently been identified to play a role in invadopodial dynamics in breast cancer. Using biosensors, RAC3 activity has been shown to localize to the invadopodial core, as well as in a ringlike structure around mature invadopodia (Donnelly et al., 2017; Rosenberg et al., 2017). Core-localized RAC3 can be activated by the GEF VAV2, which localizes to invadopodia by binding to phosphorylated cortactin, whereas  $\beta$ -PIX activates RAC3 in the ringlike region (Donnelly et al., 2017; Rosenberg et al., 2017). These interactions are proposed to promote integrin signaling and actin polymerization and thereby stimulate invadopodial maturation, matrix degradation, and cell invasion (Donnelly et al., 2017; Rosenberg et al., 2017).

**Rac-specific DOCK family GEF complexes.** Different DOCK family GEFs activate RAC(1,2,3) and/or CDC42, but not other Rho family GTPases (Gadea and Blangy, 2014). Here we describe three DOCK GEFs that act as part of complexes to activate Rac at specific locations in cells (Fig. 3 D).

**DOCK1.** DOCK1 (also known as DOCK180) is a Rac-specific GEF that forms multiple different protein complexes (Fig. 3 D). The best known DOCK1-binding protein is ELMO, and this complex localizes to lamellipodia, where it promotes migration by activating RAC1 (Grimsley et al., 2004). The ELMO–DOCK1 complex causes cytoskeletal rearrangements upon its recruitment to diverse ELMO-interacting proteins at the plasma membrane, such as the Rho GTPase RHOG or the heterotrimeric G protein subunits G $\alpha$ i2 or G $\beta$ y (Kato and Negishi, 2003; Li et al., 2013; Hernández-Vásquez et al., 2017). ELMO interacts with G $\alpha$ i2 at the membrane of breast cancer cells upon stimulation of the GPCR CXCR4, and results in

DOCK1-dependent RAC1 and RAC2 activation, migration, and invasion (Li et al., 2013). In endothelial cells, ELMO–DOCK1 associates with both the GPCR GPR124 and G $\beta$ y in a complex including the CDC42-selective RhoGEF Intersectin (Hernández-Vásquez et al., 2017). This complex promotes RAC1 and CDC42 activation and lamellipodial and filopodial extensions.

ELMO–DOCK1 also mediates cross talk between Rac and the Arf family of small GTPases, which are involved in membrane trafficking and thereby contribute to cell migration (Casalou et al., 2016). For example, the Arf family GTPase ARL4A targets ELMO–DOCK1 to membranes to activate RAC1-mediated membrane ruffling (Patel et al., 2011). In addition, DOCK1 forms a complex with the ARF6 GEF cytohesin 2 and the adaptor protein GRASP, which facilitates recruitment of DOCK1 to the plasma membrane and promotes RAC1-dependent cell migration (White et al., 2010; Koubek and Santy, 2016). These results show how important Arf-mediated membrane trafficking is for spatial activation of RAC1 at the plasma membrane.

Similarly to  $\beta$ -PIX, DOCK1 can localize to focal adhesions. However, unlike  $\beta$ -PIX, whose focal adhesion recruitment is mediated via GIT1 or GIT2 (Zhou et al., 2016), DOCK1 is targeted to focal adhesions through a different adaptor protein, CRK, which consists of one SH2 and two SH3 domains (Fig. 3 D). After integrin stimulation, DOCK1 binds to the N-terminal SH3 domain of CRK, and the CRK SH2 domain binds to tyrosine-phosphorylated p130Cas (BCAR1), which localizes to focal adhesions (Kiyokawa et al., 1998b). DOCK1 can then stimulate RAC1 activity, leading to cell spreading and migration (Kiyokawa et al., 1998a; Li et al., 2003).

ANKRD28 is a large scaffolding protein with 26 ankyrin repeats that binds to DOCK1 as well as several other proteins including protein phosphatases (Vincent et al., 2016). ANKRD28 appears to act via DOCK1 to alter focal adhesion distribution and cell migration (Tachibana et al., 2009). Interestingly, ANKRD28 and ELMO have been shown to compete for DOCK1 binding, and it has therefore been proposed that DOCK1 can promote migration via distinct mechanisms depending on its binding partner (Tachibana et al., 2009).

The diverse complexes formed by DOCK1 highlight the key role that its binding partners play in influencing its localization and activation in the context of Rac-mediated migration (Fig. 3 D).

**DOCK5.** Similarly to DOCK1, DOCK5 is a Rac-specific DOCK (Laurin and Côté, 2014). Like DOCK1, DOCK5 can localize via the p130Cas–CRK complex to focal adhesions. However, as recently demonstrated, recruitment of DOCK5 to this complex differs from DOCK1 in that it is negatively regulated by GIT2 (Frank et al., 2017). DOCK5 localizes to focal adhesions upon depletion of GIT2 or treatment with inhibitors of myosin II or ROCK, indicating that Rho-mediated actomyosin contractility is required for this effect. Notably, suppression of DOCK5 expression reduced breast cancer cell line metastasis in vivo (Frank et al., 2017).

**DOCK3.** Melanoma migration has been shown to be promoted by the Rac-specific GEF DOCK3 upon formation of a complex with the adaptor protein NEDD9 (Sanz-Moreno et al., 2008).

In summary, despite their shared ability to activate RAC1 and/or CDC42, the Dbl and DOCK family GEFs described influence migration in different ways (Fig. 3). In some cases, these differences are related to cell type-specific functions—for example, in epithelial cells that need to maintain cell–cell

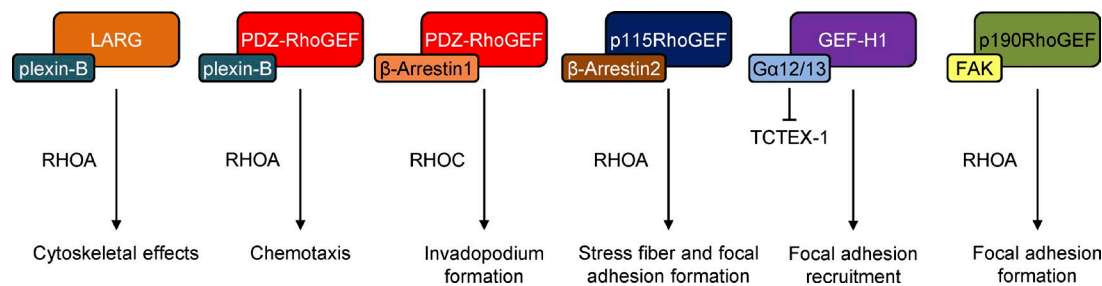


Figure 4. **Rho-specific GEF complexes involved in migration and invasion.** The Rho-specific GEFs LARG, PDZ-RhoGEF, p115RhoGEF, GEF-H1, and p190RhoGEF regulate migration in different ways depending on the complexes they form. GEFs and GEF-binding proteins are shown in arbitrarily colored boxes. For each complex, the downstream signaling targets are shown as well as the functional outcome. See text for further details.

junctions. Nevertheless, in response to different stimuli, the ability of different GEFs to form distinct protein complexes is essential in defining the functional outcome. Indeed, GEF-containing complexes appear to fine-tune RAC1/CDC42 activity by dictating their spatial and temporal localization. In addition, GEF complexes can allow Rac/CDC42 to be either directly or indirectly coupled to specific effectors. Hence, the ability of Rac/CDC42-specific GEFs to form complexes is crucial to their ability to regulate the diverse Rac/CDC42-dependent subcellular functions associated with migration.

**Rho-specific GEF complexes.** Similarly to Rac/CDC42, a number of Rho-selective GEFs (all of which belong to the Dbl family) have been shown to be involved in forming complexes that regulate migration (Fig. 4). Most of these GEFs have the potential to activate the closely related RHOA, RHOB, and RHOC proteins (Fig. 2), although in the majority of cases they have been tested only on RHOA (Schaefer et al., 2014). RHOA is often active at both the front and rear of migrating cells, and promotes actomyosin contractility through its effector ROCK, as well as actin polymerization via formin family actin nucleators (Ridley, 2015). Rho/ROCK-driven actomyosin contractility is particularly important in bleb-based cell migration (Paluch and Raz, 2013), and induces actin stress fibers and integrin-based focal adhesions in some adherent cell types (Fig. 1; Ridley, 2001).

The Rho-selective GEFs involved in cell migration as part of signaling complexes include the three closely related regulator of G protein–signaling homology domain–containing GEFs, ARHGEF12 (LARG), ARHGEF11 (PDZ-RhoGEF), and ARHGEF1 (p115RhoGEF), as well as ARHGEF2 (GEF-H1) and its relative ARHGEF28 (p190RhoGEF or Rgnef), which both have a C1 domain (Table S1 and Fig. 4; Krendel et al., 2002; Dubash et al., 2007; Iwanicki et al., 2008; Miller et al., 2014). C1 domains often bind to the membrane lipid diacylglycerol (Blumberg et al., 2008), but in the case of GEF-H1 the C1 domain appears to mediate its interaction with microtubules (Yoshimura and Miki, 2011).

**LARG, PDZ-RhoGEF, and p115RhoGEF.** Through their common regulator of G protein–signaling homology domain, LARG, PDZ-RhoGEF, and p115RhoGEF can bind and be activated by  $G\alpha_{12/13}$  heterotrimeric G protein subunits (Aittaleb et al., 2010). Depletion of all three of these GEFs prevents migration in response to thrombin, which acts via its GPCR to activate  $G\alpha_{12/13}$  (Mikelis et al., 2013). In addition to  $G\alpha_{12/13}$  subunits, other complexes have been shown to promote LARG-, PDZ-RhoGEF-, or p115RhoGEF-mediated cytoskeletal changes. For example, plexin-B transmembrane receptors bind to the PDZ domains of LARG and PDZ-RhoGEF (Perrot et al., 2002).

In the case of PDZ-RhoGEF, this interaction contributes to endothelial cell chemotaxis and angiogenesis (Basile et al., 2004).

PDZ-RhoGEF has also been implicated in activating RHOC to promote invadopodium formation, by binding to ARRB1 ( $\beta$ -Arrestin1) upon stimulation with endothelin 1 in ovarian cancer cells (Semprucci et al., 2016). On the other hand, PAK4-mediated maturation of invadopodia in melanoma cells has been linked to inhibition of PDZ-RhoGEF and a reduction of RHOA activity, although RHOC was not tested (Nicholas et al., 2016).

The subcellular localization of p115RhoGEF is influenced by the formation of a complex with ARRB2 ( $\beta$ -Arrestin2), which sequesters p115RhoGEF to the cytosol until  $\beta_2$ -adrenergic GPCR stimulation induces its translocation to the plasma membrane, where it can activate RHOA to promote stress fiber and focal adhesion assembly (Ma et al., 2012).

**GEF-H1.** GEF-H1 activates RHOA to increase actomyosin contractility and contributes to migration in a variety of cell types (Nalbant et al., 2009; Heasman et al., 2010; Fine et al., 2016). This GEF is inactive when bound to microtubules (Krendel et al., 2002), and is activated by heterotrimeric G protein subunits produced upon stimulation of GPCRs. This results in the disruption of an inhibitory complex between GEF-H1 and the dynein motor light-chain TCTEX-1, and leads to the translocation of GEF-H1 from microtubules to the cytoplasm or adhesion complexes (Meiri et al., 2014). GEF-H1 can also be recruited to adhesion complexes as a result of external tension on integrins via a pathway involving FAK, Ras, and the mitogen-activated protein kinase ERK (Guilluy et al., 2011).

**p190RhoGEF.** The formation of focal adhesions can be initiated by p190RhoGEF when it binds to FAK. This increases RHOA activity and contributes to the migration of fibroblasts (Lim et al., 2008). A GEF-independent scaffolding role for p190RhoGEF in mediating FAK localization to early adhesions has also been reported (Miller et al., 2013).

As well as acting on RHOA, p190RhoGEF can stimulate RHOC. For example, in EGF-stimulated migrating breast cancer cells, p190RhoGEF couples RHOC activation to decreased cofilin activity at regions 1–3  $\mu$ m behind the leading edge (Bravo-Cordero et al., 2013). In contrast, the RhoGAP protein ARHGAP35 (p190ARhoGAP) localizes directly to the leading edge and has the opposite effects on RHOC and cofilin activity. Hence, p190RhoGEF and p190ARhoGAP affect the spatial dynamics of actin protrusions during migration by regulating where RHOC is active (Bravo-Cordero et al., 2013). It is likely that FAK interaction with p190RhoGEF mediates its effects on RHOC, although the protein complex involved in localizing p190ARhoGAP in this context is not known.



Interestingly, p190RhoGEF and p190ARhoGAP have also been implicated in regulating RHOC activity in invadopodia (Bravo-Cordero et al., 2011).

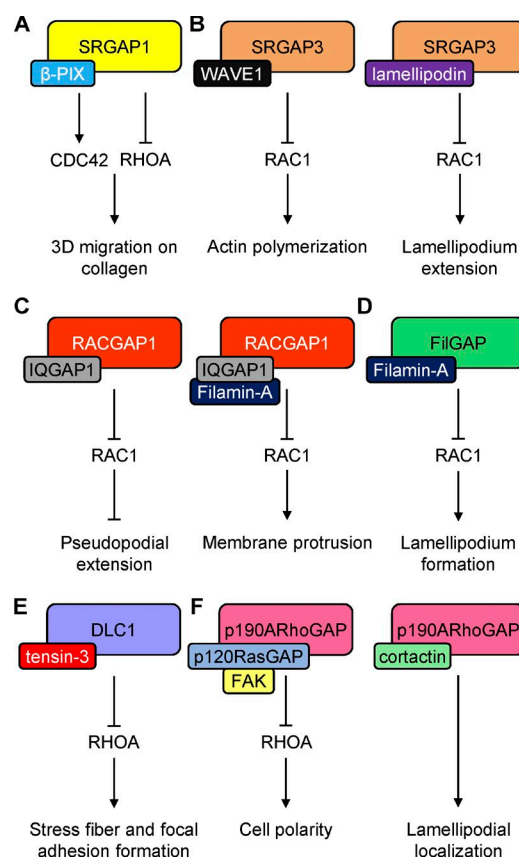
### GAP complexes

RhoGAPs (Table S2), which inactivate Rho GTPases, are less studied overall compared with RhoGEFs. Nevertheless, given the interconnected nature of Rho GTPase signaling networks, several RhoGAPs have been shown to contribute to cell migration in more ways than by merely terminating Rho GTPase signals, as already described above (in the  $\beta$ -PIX section) for SRGAP1 (Fig. 5 A; Kutys and Yamada, 2014). In this section, we will describe RhoGAP-containing complexes that have been identified to regulate migration (Fig. 5).

**Rac-specific GAP complexes. SRGAP3.** Like SRGAP1, SRGAP3 is a member of the Slit-Robo family of RhoGAP proteins, which have been linked to the migration of neuronal cells (Coutinho-Budd et al., 2012). SRGAP3 has been proposed to locally deactivate RAC1 activity at the leading edge of migrating cells through its interactions with lamellipodium-associated components (Fig. 5 B). For example, SRGAP3 attenuates RAC1-mediated neurite outgrowth by binding to the WASF1 (WAVE1/Scar) complex, which is itself a RAC1 effector (Soderling et al., 2002). Similarly, the VASP-binding protein RAPH1 (lamellipodin) has been identified to recruit SRGAP3 to protrusions where it can inhibit lamellipodium formation (Endris et al., 2011), presumably acting as a negative feedback loop to restrict the action of lamellipodin in stimulating lamellipodia extension (Carmona et al., 2016). SRGAP3 can also localize to focal complexes and appears to destabilize these structures (Yang et al., 2006; Endris et al., 2011), perhaps through its interactions with SH3 domains of proteins involved in endocytosis (Wuertenberger and Groemping, 2015). SRGAP3 has been suggested to act as a tumor suppressor in mammary epithelial cells, possibly because of its negative effect on migration and invasion (Lahoz and Hall, 2013).

**RACGAP1.** Although best known for having an essential role in cytokinesis (Zuo et al., 2014), the RAC1-specific GAP RACGAP1 has also been linked to migration and invasion. Under conditions that induce  $\alpha_5\beta_1$  integrin recycling in an ovarian cell line migrating in 3D, AKT1-mediated phosphorylation of RACGAP1 results in its recruitment to IQGAP1 at the tips of invasive pseudopods. Here, it can inhibit RAC1 activity, and this was found to drive invasion by enhancing RHOA activity through an undefined mechanism (Jacquemet et al., 2013a). Upon integrin stimulation, RAC1-mediated protrusions could also be inhibited by RACGAP1 after its recruitment to a complex of IQGAP1 and the actin filament cross-linker and scaffold protein Filamin-A (Fig. 5 C; Jacquemet et al., 2013b).

**FilGAP.** Another RAC1-specific GAP that interacts with Filamin-A is ARHGAP24 (FilGAP). FilGAP is activated by the RHOA effector ROCK and deactivates RAC1 to reduce lamellipodium formation (Fig. 5 D; Ohta et al., 2006). Hence, FilGAP is involved in regulating cross talk between RHOA and RAC1. An example of a functional outcome of this cross talk is mesenchymal-amoeboid transition. Mesenchymal tumor cells migrate with an elongated, RAC1-dependent morphology but can switch to a rounded Rho/ROCK-driven amoeboid morphology (Fig. 1). By inhibiting RAC1 downstream of RHOA, FilGAP has been implicated in regulating mesenchymal to amoeboid transition in carcinoma cells (Saito et al., 2012). Despite not binding to Filamin-A (Mori et al., 2014), a FilGAP-related



**Figure 5. GAP complexes involved in migration and invasion.** The RhoGAPs SRGAP1 (A), SRGAP3 (B), RACGAP1 (C), FilGAP (D), DLC1 (E), and p190ARhoGAP (F) regulate migration in different ways depending on the complexes they form. GAPs and GAP-binding proteins are shown in the complexes they form. For each complex, the downstream signaling targets are shown as well as the functional outcome. See text for further details.

protein, ARHGAP22, also controls the mesenchymal/amoeboid switch in melanoma cells (Sanz-Moreno et al., 2008).

**Rho-specific GAP complexes. DLC1.** DLC1 (deleted in liver cancer 1) is a tumor suppressor that has been implicated in regulating RHOA activity in the context of migration (Braun and Olayioye, 2015). DLC1 can localize to focal adhesions, where it interacts with tensin proteins. Tensins 1–3 are actin-binding proteins that link actin filaments to integrins (Haynie, 2014). The binding of tensin-3 overcomes autoinhibition of DLC1 GAP activity and allows it to decrease RHOA activity and reduce migration (Fig. 5 E; Cao et al., 2012). Upon stimulation of epithelial cells with EGF, this interaction is disrupted and DLC1 instead binds to phosphatase and tensin homologue (PTEN), which does not activate DLC1, therefore increasing RHOA activity at the cell rear (Cao et al., 2015). Concomitantly, tensin-3 switches places with PTEN and interacts with phosphatidylinositol 3-kinase at the leading edge. This removal of PTEN-mediated phosphatidylinositol 3-kinase inhibition results in RAC1 activity, presumably through activation of an unidentified GEF (Cao et al., 2015). Hence, this intriguing pathway demonstrates that binding partner-dependent DLC1 activity is capable of regulating polarized migration by defining the spatiotemporal activation of RHOA.

**p190ARhoGAP.** RHOA activity in migrating cells can also be controlled by p190ARhoGAP. As described in the



p190RhoGEF section, this GAP inhibits RHOC activity at the leading edge of breast cancer cells in response to EGF stimulation (Bravo-Cordero et al., 2013). A variety of extracellular stimuli induce SRC family tyrosine kinases to phosphorylate and activate p190ARhoGAP, thereby reducing RHOA activity. Depending on the cell type, stimulus, and conditions, this can either increase or decrease cell migration (Arthur et al., 2000; Arthur and Burridge, 2001; Bartolomé et al., 2014). For example, although GPCR activation of  $G\alpha_{12/13}$  normally activates RHOA (see the GEF-H1 section above), in melanoma cells  $G\alpha_{13}$  associates with the SRC family kinase BLK, which tyrosine-phosphorylates p190ARhoGAP upon CXCR4 stimulation and thereby inhibits RHOA (Bartolomé et al., 2014).

FAK can also phosphorylate p190ARhoGAP, and together these proteins form a complex with the Ras family GAP RASA1 (p120RasGAP) that localizes to leading edge focal adhesions. The formation of this complex is proposed to promote cell polarity by locally inhibiting RHOA activity (Fig. 5 F; Tomar et al., 2009). Recently, p190ARhoGAP has been shown to be recruited to membrane protrusions through an interaction with cortactin. This binding is mediated by a region of p190ARhoGAP termed the polarization localization sequence, which appears to have an autoinhibitory effect on p190ARhoGAP function. The mechanism through which this autoinhibition is overcome is unclear but, intriguingly, cancer-associated mutations in this region were shown to affect p190ARhoGAP activity and localization, and to impair the directionality of migrating cells (Binamé et al., 2016). Binding of the Rho GTPases RND3 and RAC1 to a similar region has also been implicated in the regulation of the related RhoGAP ARHGAP5 (p190BRhoGAP; Wennerberg et al., 2003; Bustos et al., 2008).

### Conclusions and future perspectives

Although the roles of Rho GTPases in regulating cell migration and invasion were established over 20 years ago, recent findings illustrate some of the complexities that underlie their regulation in response to different stimuli and environments. The ability of RhoGEFs and RhoGAPs to form complexes with diverse proteins, including signaling molecules, cytoskeletal or focal adhesion components, scaffolds, or even Rho GTPase effectors themselves, is emerging as a key factor in the regulation of spatiotemporal Rho GTPase activation in migration and invasion. Individual GEFs and GAPs can localize differently depending on which proteins they form complexes with, highlighting the importance of these distinct signaling units in defining functional outcomes.

An area that requires more investigation is the potential for other Rho GTPase signaling components, especially RhoGDIs and the lesser-studied “atypical” Rho GTPases (Fig. 2), to form complexes that contribute to migration. Moreover, given the evidence of cross talk between Rho GTPases in migration, future studies should aim to further characterize the potential for interacting GEF and GAP pairs to regulate combinations of Rho GTPases in response to specific stimuli. These interactions should also be probed more extensively in the context of invasion, especially with respect to invadopodium dynamics. Untangling the interconnected roles of Rho GTPases, GEFs, GAPs, and effectors in cancer cell invasion is necessary so that effective therapeutic strategies can be developed against these targets.

Finally, so far most studies on the roles of GEF and GAP complexes in cell migration have been performed in vitro. To

understand whether these complexes also contribute to cell migration and invasion in vivo, future work should focus on studying these protein–protein interactions in a variety of complementary in vivo models, as well as comparing their levels in normal and diseased human tissues.

### Online supplemental material

Table S1 shows Dbl and DOCK family RhoGEFs. Table S2 shows RhoGAPs.

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